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(54) Title: POLYPEPTIDE COMPOUNDS THAT FORM β SHEETS

(57) Abstract

The present invention is directed to a polypeptide compound having the following formula: $J_nA_{m1}X_uA_{m2}J_n$, where A is either a D- or L-alanine amino acid and m1 or m2 are 0 to about 40 with the proviso that m1 + m2 is 10 to about 40. J is a charged amino acid and n is 1 or 2. X is any amino acid except proline and u is 0 or 1. Such polypeptides form β sheets in an aqueous environment and can be used to hydrolyze compounds.

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POLYPEPTIDE COMPOUNDS THAT FORM β SHEETS

The invention was made in part with government support under Grant No. GM45583 awarded by the National Institutes of Health. The Government has certain rights in the invention.

The present invention relates to a polypeptide that forms β sheets soluble in an aqueous environment and methods of using the peptide to hydrolyze compounds.

BACKGROUND OF THE INVENTION

A polypeptide is a chain of amino acids linked together by peptide bonds. A polypeptide forms secondary structures described by the angles of rotation of bonds that interconnect the amino acids in the polypeptide. These are the bonds in one amino acid between the nitrogen and α carbon, designated the phi (ϕ) bond, the α carbon and the carbonyl carbon, designated the psi (ψ) bond, and in the peptide bond between the carbonyl carbon and the nitrogen of the adjacent amino acid. Regular secondary structures occur when all the ϕ bond angles in a polypeptide segment are equal to each other and all the ψ bonds are equal to each other.

The α helix and β sheet for a polypeptide are generally the most thermodynamically stable of the regular secondary structures. Such structures are geometrically defined by the number of amino acid residues per 360° turn of the helix and the distance between α carbon atoms of adjacent amino acids measured parallel to the axis of the helix (d). The helix pitch (p) measures the distance between repeating turns of the helix on a line parallel to the helix axis and is defined as

$$p = d \times n.$$

In a β sheet, a polypeptide or polypeptide segment are in an extended helix having an n = 2. The strand of one polypeptide segment is hydrogen bonded to other strands in the β structure conformation and thus β structure depends upon intermolecular as well as intramolecular interactions. The polypeptide segments in a β structure can be aligned either in parallel or antiparallel direction to its neighboring segments. In the parallel form, the β sheet has φ and ψ bonds of approximately -119° and +113°, respectively. For the antiparallel form, the β sheet has φ and ψ bonds of approximately -139° and +135°, respectively. The β sheet is termed a sheet because large numbers of polypeptide segments interhydrogen bonded together give a pleated sheet appearance. The side chain groups are projected above and below the planes generated by the hydrogen-bonded polypeptide chains. A large number of β sheets can aggregate to form a very large, or macro, β sheet.

Generally, one cannot predict with certainty that a polypeptide of a given amino acid sequence will form a β sheet because minor alterations in the amino acid sequence can effect whether or not a β sheet will form. Moreover, regular secondary structure depends on many parameters in addition to the amino acid sequence including, for example, solution conditions. However, it is known that synthetic polypeptides containing alternating charged and hydrophobic amino acids tend to form β sheets. In particular, poly(Val-Lys), poly(Leu-Lys), poly(Lys-Phe), poly (Tyr-Lys), poly(Glu-Ala) and poly(Glu-Tyr) form β sheets. Further, oligopeptides(Val-Glu-Val-Lys) and (Val-Glu-Val-Orn) form β sheets. Such polypeptides form β sheets composed of two polypeptides because the like-charge of the alternating charged amino acids repel other polypeptides.

Polypeptides containing a copolymer block of 20 valine amino acids flanked on either end by a sequence of 10 alternating D- and L-lysine residues are also known to form β sheets. Such polypeptides can form β sheets composed of more than two polypeptides because the hydrophobic core allows for such aggregation. However, the large number of like-charged lysine groups on the ends of such polypeptides limits the size of β sheets that are formed and very high salt concentration is required for aggregation. Interestingly, similar polypeptides containing a copolymer block of alanine are reported to form α helix sheets and hairpin-like turns rather than β sheets.

An amphiphilic polypeptide has been described that forms a macro β sheet. In such a polypeptide, the polypeptide is 16 amino acids long and every other amino acid is alanine, with alternating glutamic acid and lysine charged residues. The alanine amino acids intermolecularly bind together and the alternating glutamic acid and lysine amino acids bind with the amino acids of the opposite charge. Such polypeptides are suggested for slow-diffusion drug-delivery systems, artificial skin, separation matrices and as enzymes having hydrolysis activity. Further such polypeptides are suggested as a drug screening assay for neurodegenerative diseases that result in the deposition of pathological amounts of a the β sheet protein amyloid.

There exists a need for new synthetic polypeptides that form β sheets, particularly macro β sheets, for bioengineering, enzymatic and drug screening applications. The present invention satisfies these needs and provides related advantages as well.

SUMMARY OF THE INVENTION

The present invention is directed to a polypeptide comprising a polypeptide that forms a β sheet in an aqueous environment having the following formula:

$$J_nA_{m1}X_uA_{m2}J_n$$

where A is either a D- or L-alanine amino acid and m1 or m2 are 0 to about 40 with the proviso that m1 + m2 is 10 to about 40. J is a charged amino acid and n is 1 or 2. X is any amino acid except proline and u is 0 or 1.

The present invention is also directed to a polypeptide comprising a polypeptide that forms a β sheet in an aqueous environment having the following formula:

$$Ac\text{-}J_nA_{m1}X_uA_{m2}J_n\text{-}NH_2$$

A is either a D- or L-alanine amino acid and m1 or m2 is 0 to about 40 with the proviso that m1 + m2 equal 10 to about 40. J is selected from the group consisting of lysine, hydroxylysine, arginine, histidine, aspartic acid, glutamic acid and γ-carboxyglutamic acid and n is 1 or 2. Ac is an acylation modification to the amino terminus and NH_2 is an amidation modification to the carboxylic acid terminus. X is selected from the group consisting of cysteine, threonine, tyrosine, and serine amino acid and u is 0 or 1.

The present invention is also directed to a composition comprising two or more of the polypeptides above in a β sheet. Further, the present invention is to a method of hydrolysis comprising contacting a compound to be hydrolyzed with the polypeptide described above.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the RP-HPLC chromatograms of (A) Ac-$KA_{14}K$-NH_2 and (B) Ac-KA_8PA_5K-NH_2.

Figure 2 shows the CD spectra in aqueous solution of (A) mean residue ellipticities of 100 μM of Ac-$KA_{14}K$-NH_2 (dashed line) or Ac-KA_8PA_5K-NH_2 (solid line) and (B) the concentration-dependent CD spectra intensity of Ac-$KA_{14}K$-NH_2 at concentrations of 1 μM (solid line), 2.5 μM (dashed line), 5 μM (dotted), and 10 μM (center line).

Figure 3 shows the CD spectra of Ac-$KA_{14}K$-NH_2 (solid line) and Ac-KA_8PA_5K-NH_2 (dotted line) with 80% TFE and Ac-$KA_{14}K$-NH_2 (dashed line) and Ac-KA_8PA_5K-NH_2 (center line) with 7mM SDS.

Figure 4 shows effect of temperature, pH and urea on the CD mean residue ellipticities of Ac-$KA_{14}K$-NH_2. (A) The CD spectrum of Ac-$KA_{14}K$-NH_2 at 5°C (solid line), 30°C (dashed line), 60°C (dotted line) and 85°C (center line) is shown. The Ac-$KA_{14}K$-NH_2 mean residue ellipticity at 216 mm is shown as a function of (B) pH, or (C) urea concentration.

Figure 5 shows the effects of mixing 200μM each Ac-$KA_{14}K$-NH_2 and Ac-KA_8PA_5K-NH_2 by 0.14μg/ml protease K as determined by RP-HPLC as a function of time.

Figure 6 shows CD spectra of 100μM Ac-$KYA_{13}K$-NH_2 in the presence (dashed line) or absence of urea (solid line).

Figure 7 shows binding of ANS to β sheet complexes. (A) The fluorescence intensity of ANS as a

function of peptide concentration to (■) Ac-$KA_{14}K$-NH_2, (●) Ac-$KEA_{13}KE$-NH_2 and (○) Ac-KA_8PA_5K-NH_2 is shown. (B) The CD spectra of ANS with Ac-$KA_{14}K$-NH_2 (solid line) or Ac-KA_8PA_5K (dashed line).

Figure 8 shows UV spectra of 5'-mononucleodides in the presence or absence of 200μM Ac-$KA_{14}K$-NH_2 for (A) d-AMP, (B) TMP, (C) d-GMP and (D) d-CMP.

Figure 9 shows the near UV CD spectra of 5'-mononucleotides in the presence or absence of 200μM Ac-$KA_{14}K$-NH_2 for (A) d-AMP, (B) TMP, (C) d-GMP and (D) d-CMP.

Figure 10 shows Ac-$KA_{14}K$-NH_2 binds and enhances hydrolysis of BDNPP. (A) The near UV CD spectra of 100μM BDNPP in the presence (solid line) or absence (dashed line) of 200μM Ac-$KA_{14}K$-NH_2 is shown. (B) Changes in the optical density (OD) at 400 nm as a function of time for 100μM BDNPP (■) in buffer, in the presence of (▾) 200μM Ac-KA_8PA_5K-NH_2 and (▴)200μM Ac-$KA_{14}K$-NH_2 are shown.

Figure 11 shows Ac-$KA_{14}K$-NH_2 binds and enhances hydrolysis of ONPG6P. (A) The near UV CD spectra of 100μM ONPG6P in the presence (■) or absence (▴) of 200μM Ac-$KA_{14}K$-NH_2 is shown.

DETAILED DESCRIPTION OF THE INVENTION

The present invention is a polypeptide that has the surprising capability of forming macro β sheets in an aqueous environment. The polypeptide is composed of a copolymer block of 10 or more alanine amino acids and one or two flanking amino acids on both ends of the copolymer block and are charged amino acids. All alanine amino acids are either the D or L-form. If two charged amino

acids are on one end of the copolymer block, the charged amino acids at that end must have opposite charge or absolute configuration. The amino and carboxylic acid termini can contain chemical modifications that do not prevent β sheet formation. The polypeptide can optionally contain a library amino acid inserted anywhere in the copolymer block or between the flanking amino acids and the copolymer block. A library amino acid can be any amino acid except one that prevents β sheet formation such as, for example, proline.

The present invention also describes a method of hydrolyzing compounds using the claimed polypeptide. In particular, the polypeptide has phosphodiesterase activity and readily hydrolyzes nucleic acids. The polypeptide also has glycosidase activity and readily hydrolyzes sugar phosphates.

Further, the present invention describes a method for screening drugs for the prevention or treatment neurodegenerative diseases, including, for example, Alzheimer's disease. In such diseases, amyloid protein deposition is a hallmark. Amyloid protein contains large amounts of β sheets. The claimed polypeptides can be used to screen for drugs which inhibit or disrupt the β sheet.

The term "polypeptide" refers to a chain of amino acids linked together by peptide bonds. Such polypeptides can be chemically synthesized. The polypeptide of the present invention requires a copolymer block of 10 or more alanine amino acids in order to form β sheets. The length of the copolymer block is limited only by its synthesis. The polypeptide has an amino and carboxylic acid terminus and, as described below, either the amino or carboxylic acid terminus or both can be chemically modified.

Amino acids are molecules having a central α-carbon to which a carboxylic acid, amine, hydrogen and side chain groups (R) are covalently bound. The R group defines the structures of the different amino acids. The term "amino acid" refers to the common amino acids and derived amino acids. A common amino acid is defined as an amino acid for which at least one specific codon exists in the DNA genetic code. Examples of common amino acids include alanine, lysine, arginine, histidine, glutamic acid, aspartic acid, cysteine, threonine, tyrosine, and serine amino acids. A derived amino acid is formed from one of the common amino acids, usually by an enzyme facilitated reaction, after the common amino acid has been incorporated into the polypeptide. Examples of derived amino acids include hydroxylysine and γ-carboxyglutamic acid.

The central α-carbon atom of an amino acid having four different substituents arranged in a tetrahedral configuration is asymmetric and exists in two enantiomeric forms. All amino acids, except glycine, exhibit such optical isomerism. The term "absolute configuration" refers to the optical isomerism of an amino acid and can be either the L or D form. A copolymer block of alanine amino acids can have either absolute configuration but must all have the same absolute configuration.

The term "charged amino acid" refers to an amino acid having an ionizable side chain group, R. The charged state of such an amino acid is determined by the acid dissociation constant. Amino acids whose ionizable side chains contain nitrogen atoms, such as, for example, lysine, arginine and histidine, have a high acid dissociation value. Such amino acids are usually in acid form and have a net positive charge at physiological pH (approximately pH 7) and are herein termed a "positively

charged" or "positive" amino acid. Amino acids whose ionizable side chain contains a carboxylic acid group, such as, for example, aspartic acid and glutamic acid, have a low acid dissociation value. Such amino acids are usually in the base form and have a negative charge at physiological pH and are herein termed a "negatively charged" or "negative" amino acid.

A charged amino acid can be either the D or L-form regardless of the alanine amino acid absolute configuration. However, if two charged amino acids are on one end of the copolymer block, the charged amino acids at that end must have opposite charge or opposite absolute configuration.

The amino and carboxylic acid terminus of the polypeptide can be chemically modified. Any chemical modification of polypeptide termini known in the art can be used so long as the chemical modification does not prevent secondary β sheet. Examples of amino terminal chemical modifications include acyl groups such as, acetyl (Ac), formyl (For), or benzl (Bzl), and protective groups, such as t-butyloxycarbonyl (t-Boc), 9-fluorenylmethyloxycarbonyl (Fmoc), and benzloxycarbonyl (CBZ). An amino terminal chemical modification replaces an amine group by the chemical modification group. In such an amino acid, the chemical modification can replace either the amino group bonded to the α-carbon or of the R group, if any. Examples of carboxylic acid terminal chemical modifications include amide (NH_2) or ethanolamine ($-NHCH_2CH_2OH$) groups. A carboxylic acid terminal chemical modification replaces a hydroxyl group of a carboxylic acid group with the chemical modification group. In such an amino acid, the chemical modification group can replace either the hydroxyl of the carboxylic acid group bonded to the α-carbon or of the R group, if any.

A library amino acid can optionally be positioned anywhere in the copolymer block or between the charged amino acids and the copolymer block. A library amino acid can be any amino acid except one that prevents β sheet formation such as, for example, proline. The library amino acid can be D or L-form regardless of the copolymer block absolute configuration. In the present invention, a library amino acid can be selected from the group consisting of cysteine, threonine, tyrosine, and serine amino acids.

The terms, "β sheet(s)" or "β sheet structure(s)," are synonymous and refer to the β sheet described above. Only a segment of a polypeptide need be able to form a β sheet for the polypeptide to be a β sheet polypeptide. The term "macro β sheet(s)," refers to β sheet structures having a molecular weight of approximately 10^4 Daltons or greater.

The term "aqueous environment" refers to any environment containing water in any of its physical states and optionally containing an additional compound(s).

The polypeptide of the present invention has the following formula:

$$J_nA_{m1}X_uA_{m2}J_n$$

where A is either a D- or L-alanine amino acid and m1 or m2 are 0 to about 40 with the proviso that m1 + m2 is 10 to about 40. J is a charged amino acid and n is 1 or 2. X is any amino acid except proline and u is 0 or 1.

The polypeptide of the present invention has the following formula:

$$B\text{-}J_nA_{m1}X_uA_{m2}J_n\text{-}O$$

where B and O are chemical modifications to the amino and carboxylic acid terminal charged amino acids of the polypeptide, respectively; n is 1 or 2 and J is a charged amino acid; m1 or m2 is 0 to about 40, m1 + m2 is 10 to about 40 and A is either a D- or L-alanine amino acid; u is 0 or 1 and X is a library amino acid is any amino acid except proline.

In an embodiment of the polypeptide described immediately above, J is selected from the group consisting of lysine, hydroxylysine, arginine, histidine, aspartic acid, glutamic acid and γ-carboxyglutamic acid. In a further embodiment, X is selected from the group consisting of cysteine, threonine, tyrosine, and serine amino acid. Another embodiment of the above polypeptide is where m1 + m2 is 10 to about 30.

The present invention includes a polypeptide having the following formula:

$$Ac\text{-}J_nA_{m1}X_uA_{m2}J_n\text{-}NH_2$$

where Ac is an acylation modification to the amino terminus and NH_2 is an amidation modification to the carboxylic acid terminus. A is either a D- or L-alanine amino acid and m1 or m2 is 0 to about 40 with the proviso that m1 + m2 equal 10 to about 40. J is selected from the group consisting of lysine, hydroxylysine, arginine, histidine, aspartic acid, glutamic acid and γ-carboxyglutamic acid and n is 1 or 2. X is selected from the group consisting of cysteine, threonine, tyrosine, and serine amino acid and u is 0 or 1.

In one embodiment of the polypeptide, J is selected from the group consisting of lysine,

hydroxylysine, arginine, histidine, aspartic acid, glutamic acid and γ-carboxyglutamic acid. In another embodiment, m1 + m2 is 10 to about 30.

The invention also includes the polypeptide immediately above having the following formula:

$$Ac\text{-}J_nA_mJ_n\text{-}NH_2$$

where m is 10 to about 40.

An embodiment of a polypeptide has the following formula:

$$Ac\text{-}KA_mK\text{-}NH_2$$

where K is D or L-lysine amino acid.

The peptides of the present invention can be made by any known method of polypeptide synthesis. For example, see Schwartz, A.M. and G. D. Fasman, Biopolymers, 15:1377-1395 (1976). A particularly useful method is simultaneous multiple peptide synthesis using p-methylbenzhydryl amine resin and standard Boc chemistry. See, for example, Houghten, R. A., Proc. Natl. Acad. Sci. USA, 82, 5131-5135 (1985). In that method, final cleavage and deprotection can be done with "low-high" HF protocol. See, for example, Houghten, R. A. et al., Int. J. Pept. Protein Res. 27, 673-678 (1986), herein expressly incorporated by reference. Peptides can be extracted with water and purified by preparative RP-HPLC. Analytical RP-HPLC can be carried out, for example, using a Vydac C_{18} column (ODS, 3μ, 5 cm x 4.6 mm) and about 0.05% TFA in acetonitrile (ACN - solvent B). The peptides can be characterized by various well known means including, for example, laser desorption time-of-flight mass spectroscopy using, for example, a Kratos

Kompact Maldi-Tof mass spectrometer. Size exclusion chromatography can be preformed, for example, on a Beckman SEC 3000 column with 60% ACN, 0.05% TFA and H_2O as the mobile phase. Such a method is particularly useful for making Ac-$J_nA_{m1}X_uA_{m2}J_n$-NH_2, Ac-KA_mK-NH_2, Ac-$KA_{14}K$-NH_2, Ac-$KEA_{13}KE$-NH_2, and Ac-$KA_{14}K$-NH_2.

Polypeptides having an amino or carboxylic acid terminus that is not chemically modified can be made by methods well known in the art. See, for example, Cuervo, J.H., et al., Peptide Research 1:81 (1988). For example, such polypeptides having a carboxylic acid terminus can be made using known methods and selecting an appropriate resin such as Boc-amino acid-PAM (phenylacetamidomethyl) resin, carrying out the first coupling step with Boc-alanine amino acid instead of Boc-lysine amino acid. For example, such polypeptides having an amine terminus can be made by the method described above and omitting the acylation step.

The presence of a β sheet can be detected by any method known for this purpose, including, ultra violet circular dichroism (CD) assays. For example, CD measurements can be conducted on a Jasco-720 CD spectropolarimeter equipped with a Neslab RTE 110 temperature controller at 25°C. The CD results can be either CD intensity (mdeg) or mean residue ellipticity [θ] in $degcm^2dmol^{-1}$. The concentration of the peptide can be determined by any known means such as, for example, quantitiative amino acid analysis.

Typically, alternating hydrophobic and charged amino acids are used to make synthetic polypeptides that form β sheets. See for example, Blondelle, S.E. et al., Biophys. J. 68:351-359 (1995), Brack, A. and L.E. Orgel, Nature 256:383-387 (1975) and DeGrado, W.F. and J.D. Lear, J. Am. Chem. Soc. 107:7684-7689 (1985). Such

polypeptides have hydrophobic and charged surfaces on each sheet. This results in two adjacent sheets being hydrophobically packed together while the hydrophilic surfaces provide solvent accessible sites.

In contrast, β sheet formation of the present polypeptide is due to the hydrophobic packing of alanine amino acids. The charged amino acids at the ends of the polypeptide are responsible for the solubility of the polypeptide in an aqueous environment. Size exclusion chromatography and centrifugal concentration through ultrafiltration indicate that the β sheets can exceed 10^5 Daltons. Such macro β sheet formation results from extended hydrophobic interactions on both faces of a β sheet. The macro β sheets form "dumb-bell" like and a micellar complexes.

The following examples are intended to more clearly illustrate the invention, but are not intended to limit the scope thereof.

EXAMPLE 1

The following data demonstrates that polypeptides of the present invention forms a β sheet. Following synthesis, crude Ac-$KA_{14}K$-NH_2 has two distinct populations of molecules. Figure 1A shows the major component is a broad peak centered at 3.2 min and a second sharp peak at 2.5 min in reverse phase, high performance, liquid chromatography, RP-HPLC. Mass spectral analysis shows the broad and sharp peak are 1310.9 and 1311.8 Daltons, respectively. The theoretical molecular weight is 1309.7 Daltons. The presence of two peaks suggests that different secondary or tertiary structures are present.

The CD spectra of Figure 2A shows that the broad peak is a β sheet and, although the data is not shown, the sharp peak is a partial α-helix. Also in Figure 2A, the CD spectra of Ac-$KA_{14}K$-NH_2 is compared to that of Ac-KA_8PA_5K-NH_2, a polypeptide containing a proline amino acid. The proline amino acid is known to disrupt β sheet formation. The CD spectra shows that the polypeptide lacking and having the proline amino acid form or do not form the β sheet, respectively.

The β sheet formed by polypeptides of the present invention is very stable. Figure 2B shows the β sheet of Ac-$KA_{14}K$-NH_2 is concentration-independent and is retained at low concentrations. This result suggests that the stability of the β sheet is probably due to the hydrophobic packing of multiple β sheets against one another. Figure 3 shows that the β sheet of Ac-$KA_{14}K$-NH_2 is stable in presence of SDS, a hydrophobic-like compound, and TFE, α-helix promoting compound. Figure 4 shows the β sheet of Ac-$KA_{14}K$-NH_2 is stable at elevated temperatures, over a wide pH range and in increasing urea concentration. The stability of the β sheet at elevated temperatures strongly suggests that hydrophobic interactions are the primary driving force in forming the β sheet. The stability of the β sheet to pH indicates that the lysine amino acids, while contributing to the solubility of the polypeptide, do not interfere in the formation of the β sheet. The stability of the β sheet at high urea concentration further indicates that the stability is due hydrophobic effects.

Similar data is obtained for such polypeptides containing an amino acid at a library position. For example, Figure 6 shows that Ac-$KYA_{13}K$-NH_2, where Y is a tyrosine amino acid, in the presence or absence of urea forms β sheets. Moreover, the presence of tyrosine does not affect the formation or solubility of the β sheet as

determined by RP-HPLC, data not shown. Likewise, polypeptides containing cysteine threonine, and serine form β sheets.

EXAMPLE 2

The following data demonstrates that polypeptides of the present invention can bind various compounds, especially nucleic acids. Figure 7A shows the fluorescence intensity of 8-anilino-1-naphthalene sulfonic acid (ANS) in the presence of Ac-$KA_{14}K$-NH_2, Ac-$KEA_{13}KE$-NH_2, where E is glutamic acid, and Ac-KA_8PA_5K-NH_2. The data shows that ANS binds to Ac-$KA_{14}K$-NH_2, Ac-$KEA_{13}KE$-NH_2, but not Ac-KA_8PA_5K-NH_2. Figure 8A and C show a hypochromic effect on the UV spectra is observed for purine mononucleotides, 2'-deoxyadenosine 5' monophosphate, d-AMP, and 2'deoxyguanosine 5'monphosphate, d-GMP, upon complexation with Ac-$KA_{14}K$-NH_2. In contrast, Figure 8B and C show no change in UV spectra for pyridine monucleotides, thymidine 5'monphosphate, TMP, and 2'deoxycytosine 5'monophosphate, d-CMP, upon mixing with Ac-$KA_{14}K$-NH_2. As shown in Figure 9A and C, the UV hypochromic effects correlate with changes in the near UV CD spectrum for d-AMP and d-GMP upon complexation with Ac-$KA_{14}K$-NH_2. Figure 9C and B show the near UV CD spectrum is effected by d-GMP but not TMP in the presence of Ac-$KA_{14}K$-NH_2.

EXAMPLE 3

The following data demonstrate that polypeptides of the present invention can hydrolyze various substrates. Bis-[p-nitrophenyl] phosphate (BDNPP) is a model substrate of phosphodiesterase activity. As shown in Figure 10A, upon complexation with Ac-$KA_{14}K$-NH_2, BDNPP is detected by near UV CD at 280 nm.

In contrast, no complexation is detected when BDNPP is combined with Ac-KA_8PA_5K-NH_2. Figure 10B shows that Ac-$KA_{14}K$-NH_2, but not Ac-KA_8PA_5K-NH_2, causes a marked hydrolysis of BDNPP as detected by 400 nm absorbance. The Ac-$KA_{14}K$-NH_2 thus has phosphodiesterase activity and is capable of hydrolyzing nucleic acids.

The substrate o-nitrophenyl B-d-galactosidase 6-phosphate (ONPG6P) is a model substrate of glycosidic hydrolysis activity. Figure 11 shows that Ac-KA_mK-NH_2,but not Ac-KA_8PA_5K-NH_2, causes a marked hydrolysis of ONPG6P as detected by UV spectroscopy.

The polypeptide of the present can also cause amine-catalyzed decarboxylation, such as, for example, decarboxylation of oxalacetate. Moreover, the polypeptide can hydrolyze phospholipids in general.

EXAMPLE 4

The polypeptides of the present invention provide a suitable model system for studying the formation and disruption of naturally occurring proteins having β sheet structures. Naturally occurring proteins frequently have β sheet structures in a hydrophobic core located in the interior of the protein. Because the polypeptides of the present invention have a hydrophobic core, they are similar to such naturally occurring proteins. In particular, the β-amyloid protein is known to have β sheet structures in a hydrophobic core. Excessive β-amyloid protein is associated with various neurodegenerative disorders, including Alzheimer's disease. The prevention or disruption of β sheets formed by the polypeptides of the present invention is a useful screen to select potential therapeutic agents for preventing or disrupting β-amyloid protein.

All references cited herein are expressly incorporated.

Although the invention has been described with reference to the presently preferred embodiments, it should be understood that various modifications can be made by those skilled in the art without departing from the invention. Accordingly, the invention is set out in the following claims.

WE CLAIM:

1. A polypeptide comprising a polypeptide that forms a β sheet in an aqueous environment having the following formula:

$$J_nA_{m1}X_uA_{m2}J_n$$

wherein:

A is either a D- or L-alanine amino acid and m1 or m2 are 0 to about 40 with the proviso that m1 + m2 is 10 to about 40;

J is a charged amino acid and n is 1 or 2; and

X is any amino acid except proline and u is 0 or 1.

2. A polypeptide comprising a polypeptide that forms a β sheet in an aqueous environment having the following formula:

$$Ac\text{-}J_nA_{m1}X_uA_{m2}J_n\text{-}NH_2$$

wherein:

A is either a D- or L-alanine amino acid and m1 or m2 is 0 to about 40 with the proviso that m1 + m2 equal 10 to about 40;

J is selected from the group consisting of lysine, hydroxylysine, arginine, histidine, aspartic acid, glutamic acid and γ-carboxyglutamic acid and n is 1 or 2;

Ac is an acylation modification to the amino terminus and NH_2 is an amidation modification to the carboxylic acid terminus;

X is selected from the group consisting of cysteine, threonine, tyrosine, and serine amino acid and u is 0 or 1.

3. The polypeptide of claim 2 having the following formula:

$$Ac\text{-}KA_mK\text{-}NH_2$$

where K is a D or L-lysine amino acid and m is 10 to about 40.

4. The polypeptide of claim 2 wherein m1 + m2 is 10 to about 30.

5. The polypeptide of claim 3 wherein m is 10 to about 30.

6. A composition comprising two or more of the polypeptides of claim 1 in a β sheet.

7. A composition comprising two or more of the polypeptides of claim 2 in a β sheet.

8. The polypeptide of claim 7 wherein the β sheet is a macro β sheet wherein the β sheet is a macro β sheet.

9. A method of hydrolysis comprising contacting a compound to be hydrolyzed with the polypeptide of claim 1 in an aqueous environment.

10. A method of hydrolysis comprising contacting a compound to be hydrolyzed with the polypeptide of claim 2 in an aqueous environment.

11. The method of claim 9 wherein the compound to by hydrolyzed is a nucleic acid.

12. The method of claim 9 wherein the polypeptide of claim 1 has phosphodiesterase activity.

13. The method of claim 9 wherein the polypeptide of claim 1 has glycosidase activity.

14. A method of hydrolysis comprising contacting a compound to be hydrolyzed with the polypeptide of claim 3 in an aqueous environment.

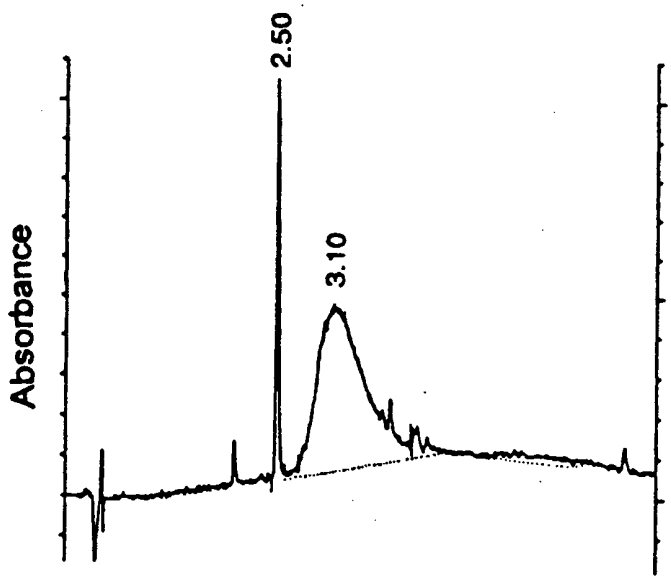
2.50
3.10
Absorbance

FIG. 1A

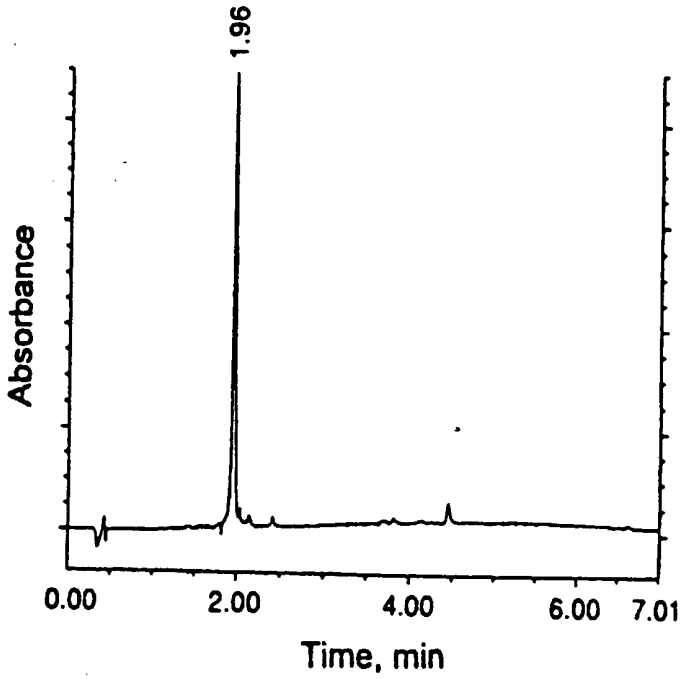
1.96
Absorbance
0.00
2.00
4.00
6.00
7.01
Time, min

FIG. 1B

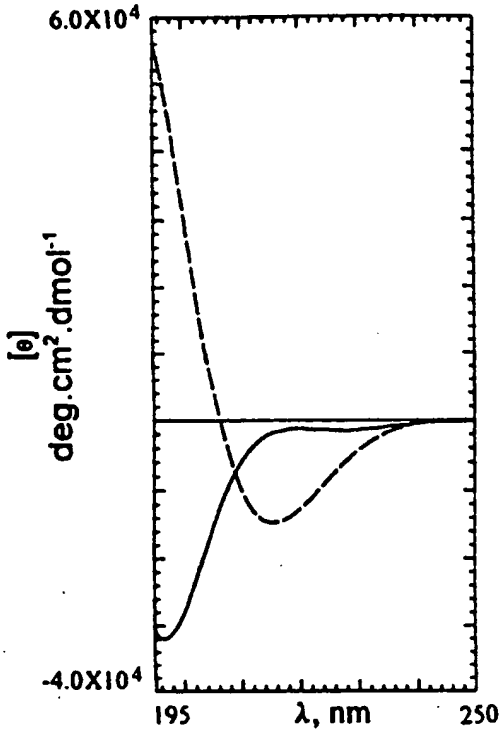

FIG. 2A

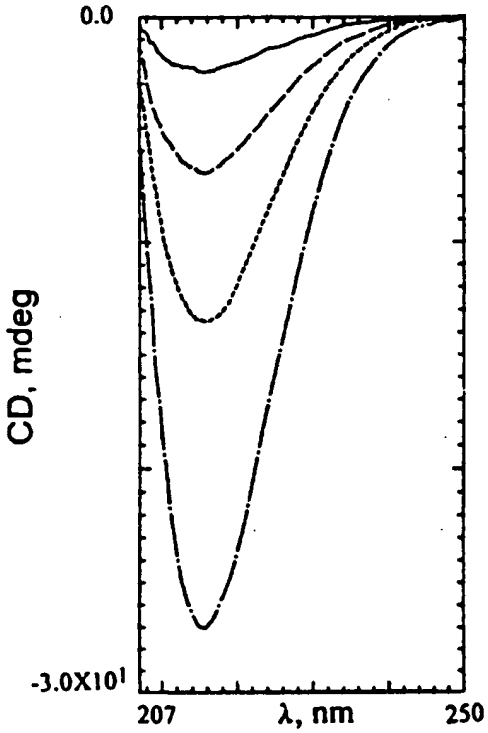

FIG. 2B

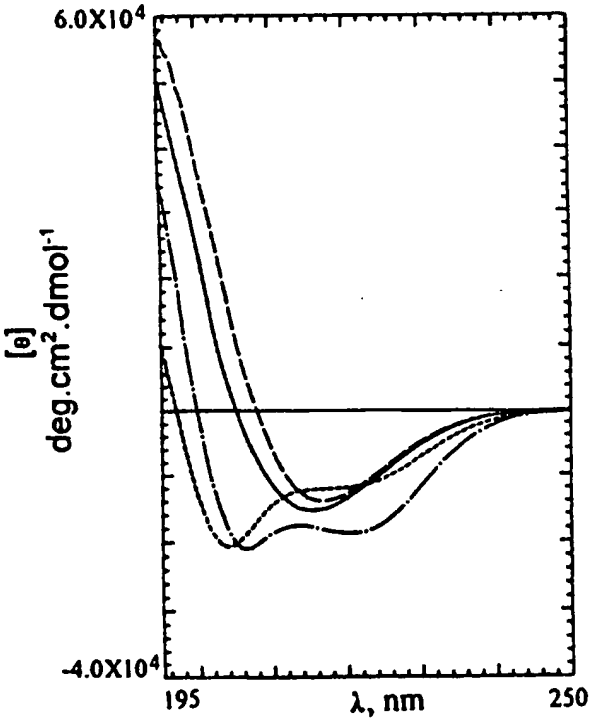

FIG. 3

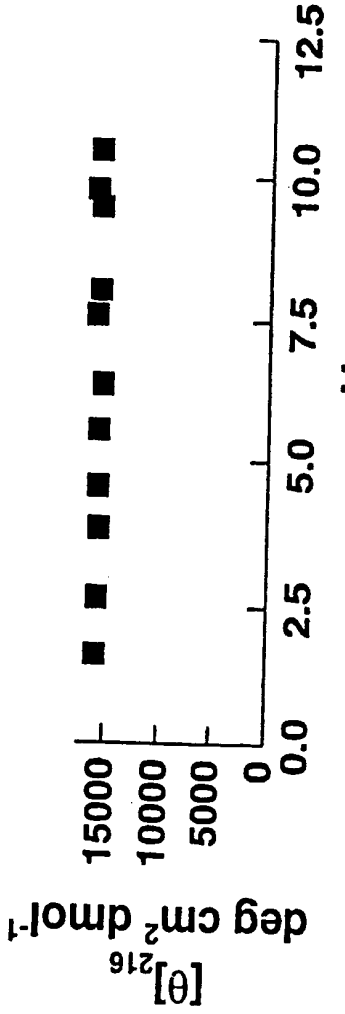

FIG. 4B

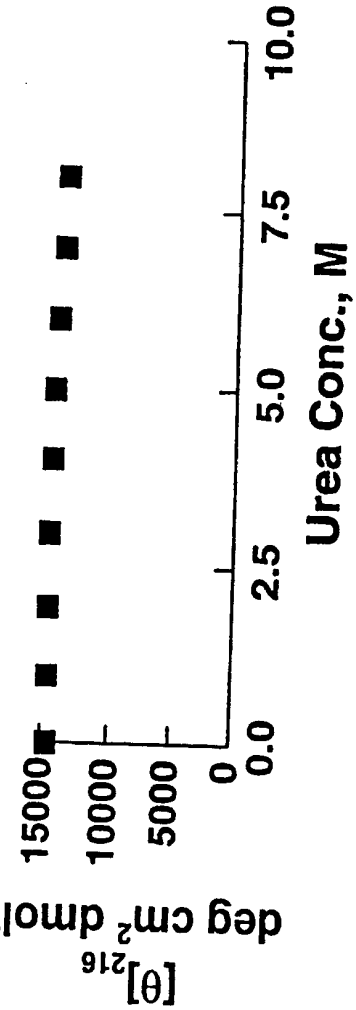

FIG. 4C

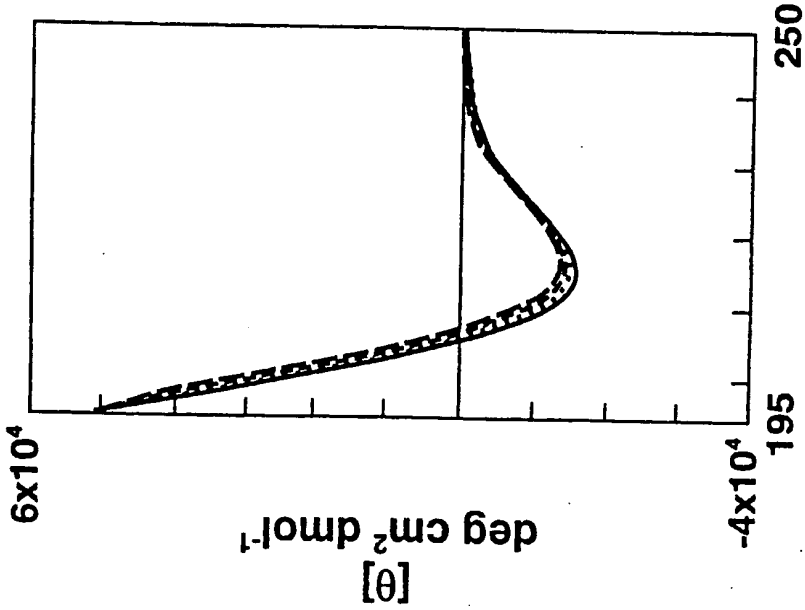

FIG. 4A

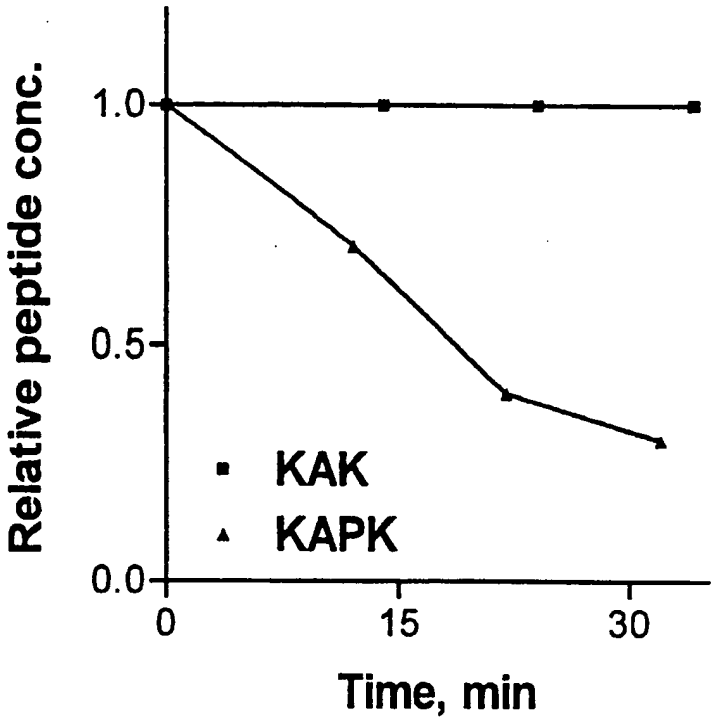

FIG. 5

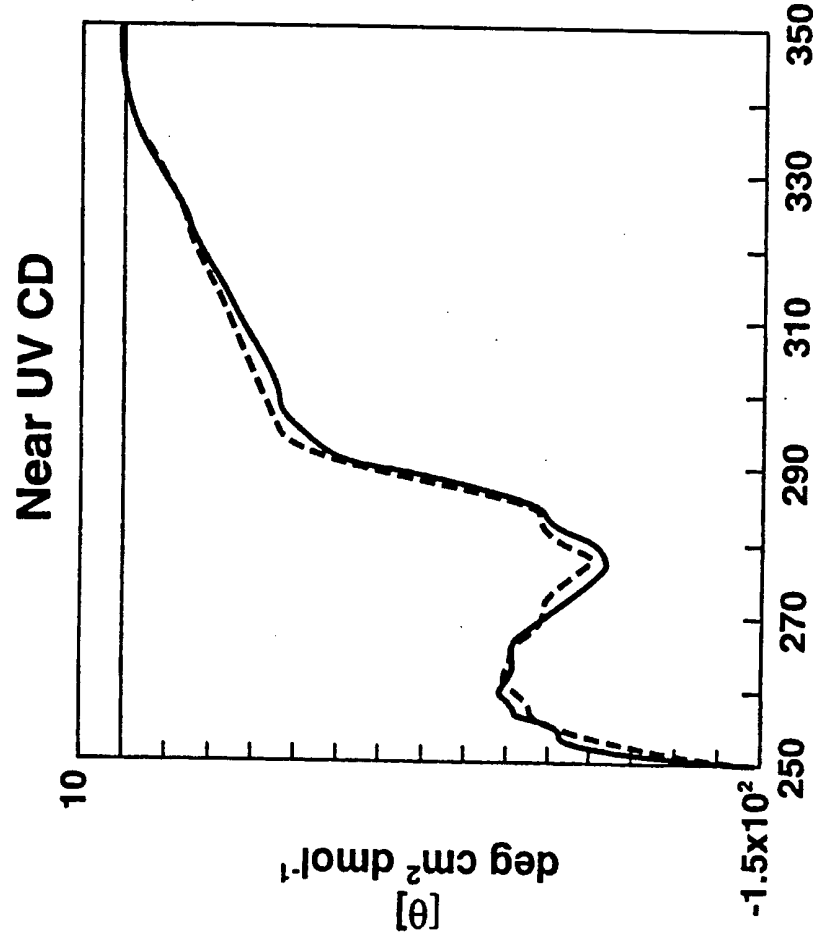
Near UV CD
10
-1.5x10²
[θ]
deg cm² dmol⁻¹
250 270 290 310 330 350
λ [nm]

FIG. 6B

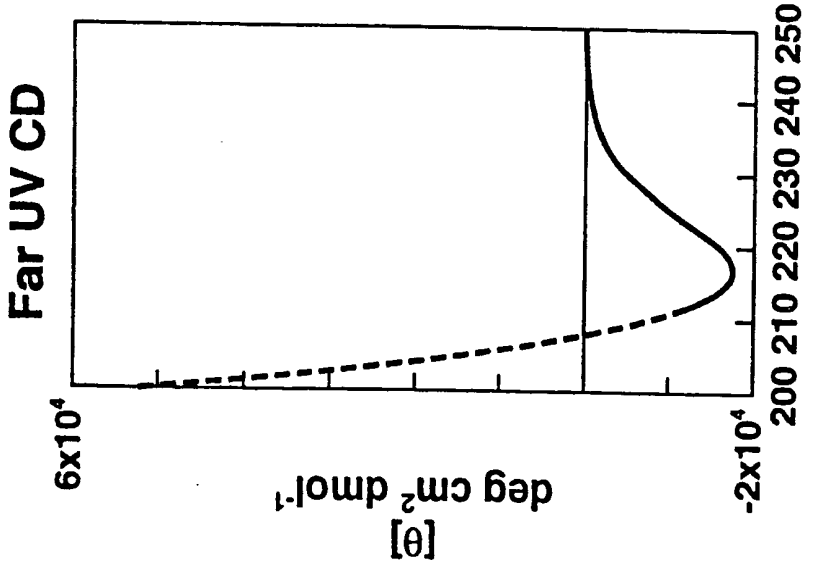
Far UV CD
6x10⁴
-2x10⁴
[θ]
deg cm² dmol⁻¹
200 210 220 230 240 250
λ [nm]

FIG. 6A

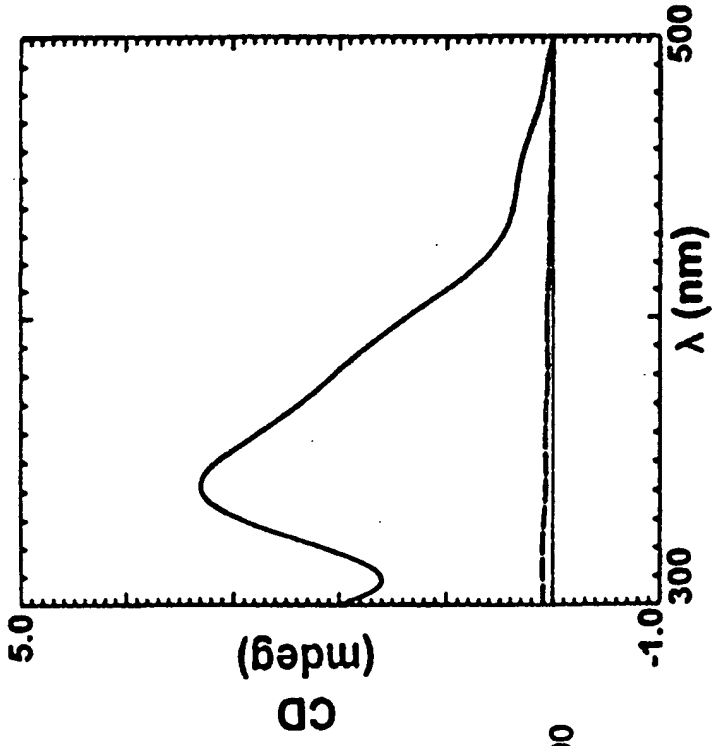
5.0
CD
(mdeg)
-1.0
300
λ (nm)
500

FIG. 7B

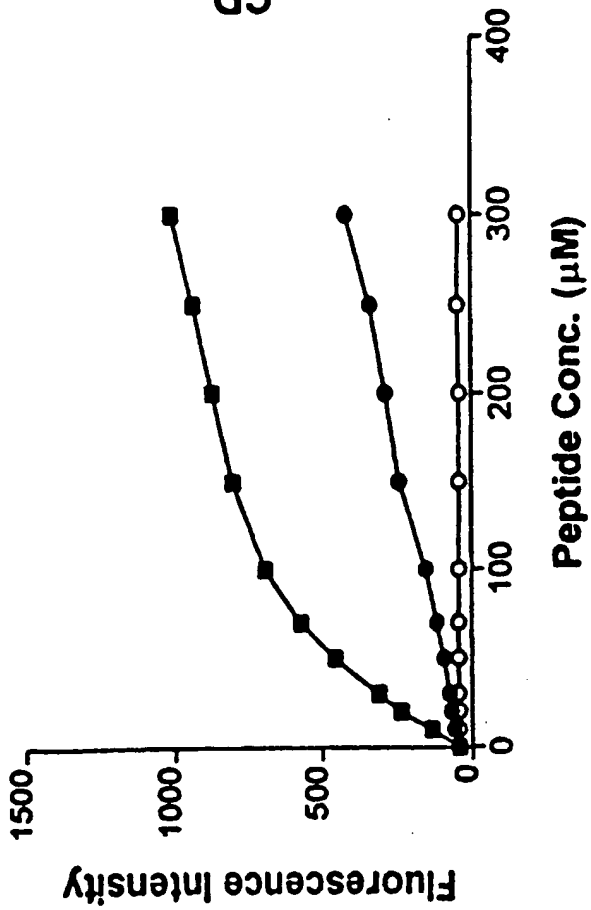
Fluorescence Intensity
1500
1000
500
0
0
100
200
300
400
Peptide Conc. (μM)

FIG. 7A

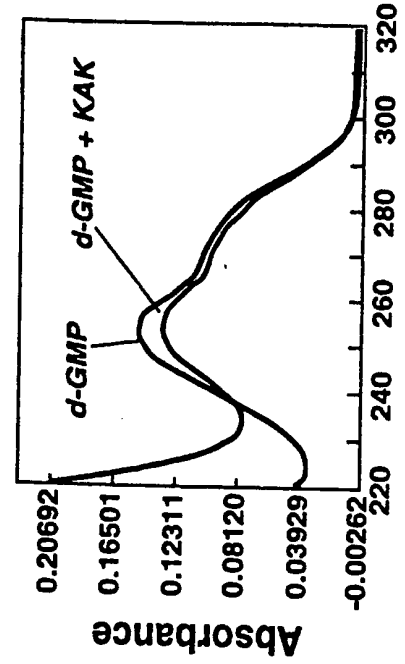

FIG. 8B

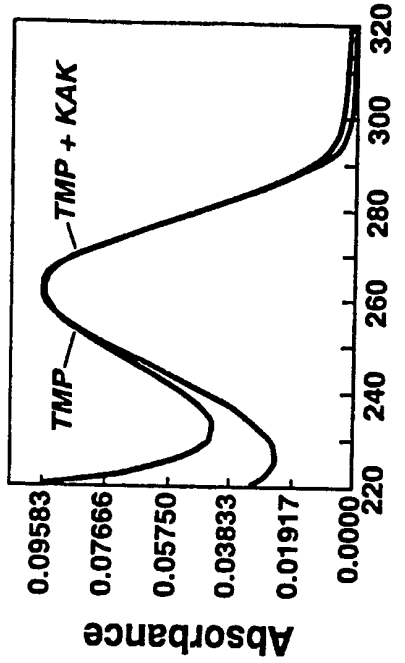

FIG. 8D

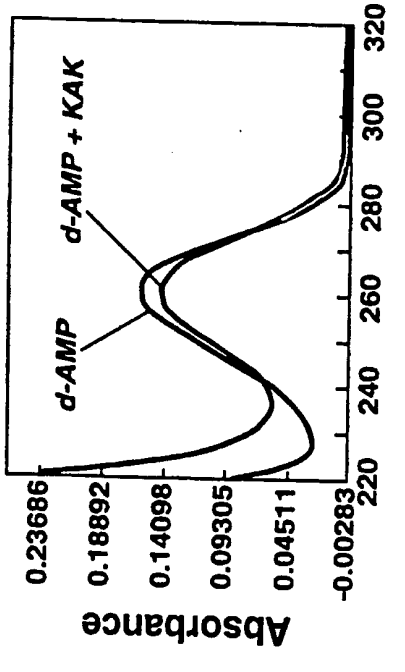

FIG. 8A

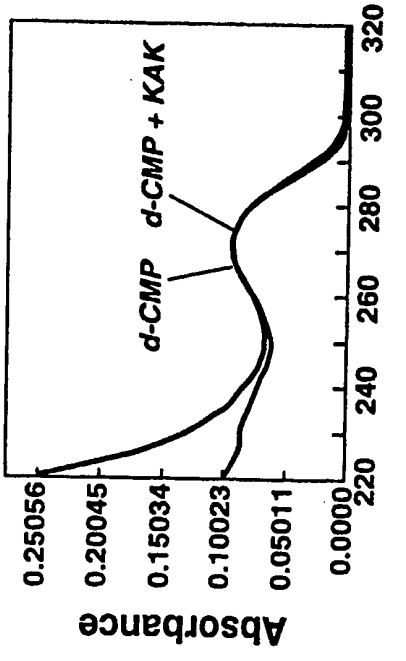

FIG. 8C

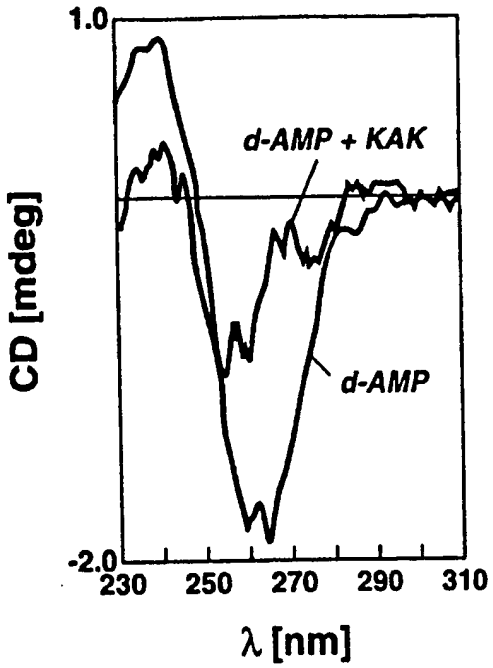

FIG. 9A

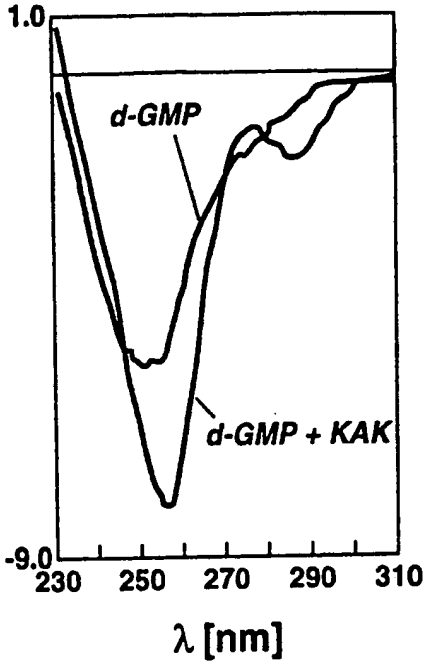

FIG. 9B

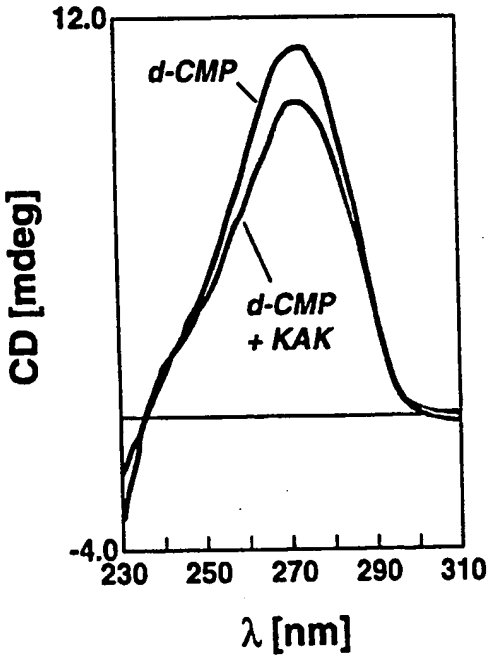

FIG. 9C

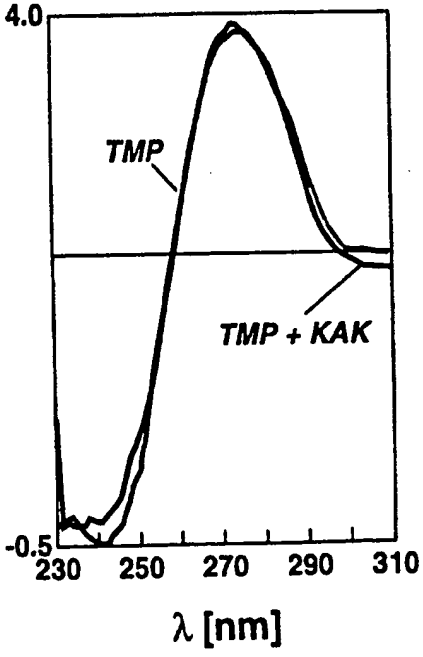

FIG. 9D

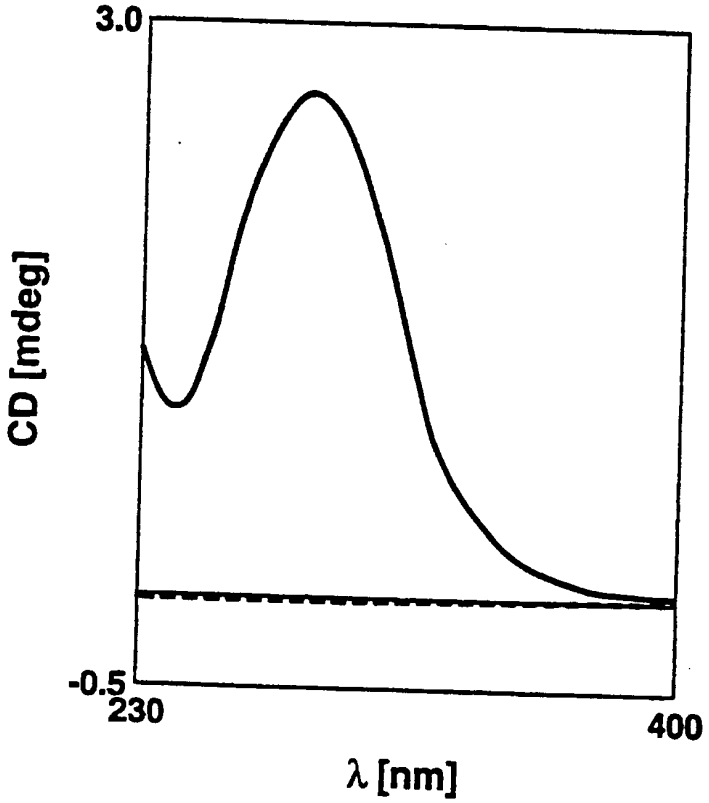

FIG. 10A

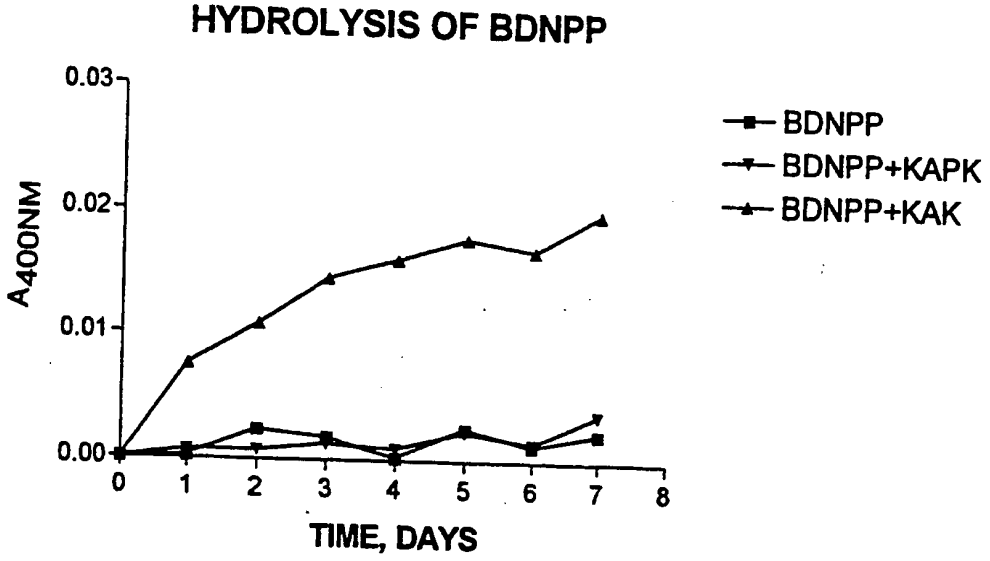

FIG. 10B

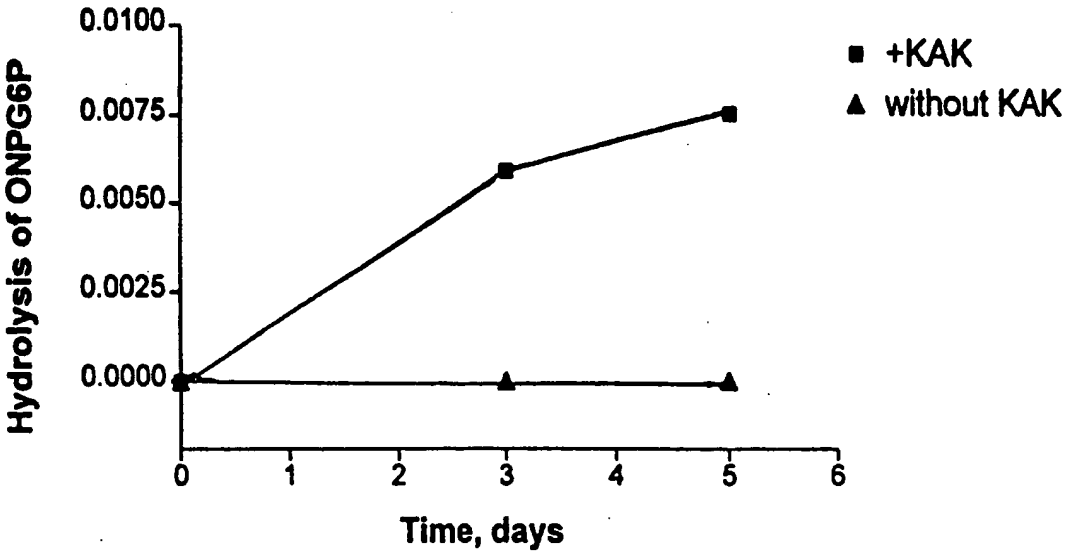
Hydrolysis of ONPG6P
0.0100
0.0075
0.0050
0.0025
0.0000
0
1
2
3
4
5
6
Time, days
+KAK
without KAK

FIG. 11

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/07564

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A61K 38/00; C07K 5/00, 7/00, 17/00
US CL :530/300, 324, 325, 326, 327
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 530/300, 324, 325, 326, 327

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, CAS ONLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Biopolymers, Volume 34, issued 1994, S. Zhang et al, "Unusually Stable beta-Sheet Formation in an Ionic Self-Complementary Oligopeptide", pages 663-672, see entire document.	1-14
Y	Proc. Natl. Acad. Sci. USA, Volume 90, issued April 1993, S. Zhang et al, " Spontaneous Assembly of a Self-Complementary Oligopeptide to Form a Stable Macroscopic Membrane", pages 3334-3338, see entire document.	1-14

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
13 AUGUST 1996	19 SEP 1996
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer AVIS M. DAVENPORT Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/07564

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Macromolecules, Volume 4, No.5, issued September 1971, A. Lewis et al, "Laser Raman Spectroscopy of Polypeptides. I. Water-Soluble Block Copolymers of L-Alanine and D,L-Lysine", pages 539-543, see entire document.	1-14